# Analysis of Breast Milk to Assess Exposure to Chlorinated Contaminants in Kazakhstan: Sources of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) Exposures in an Agricultural Region of Southern Kazakhstan

Kim Hooper,<sup>1</sup> Tamara Chuvakova,<sup>2</sup> Gulnara Kazbekova,<sup>3</sup> Douglas Hayward,<sup>4</sup> Asel Tulenova,<sup>5</sup> Myrto X. Petreas,<sup>1</sup> Timothy J. Wade,<sup>1</sup> Kathleen Benedict,<sup>1</sup> Ying-Yin Cheng,<sup>1</sup> and Jean Grassman<sup>6</sup>

<sup>1</sup>Hazardous Materials Laboratory, California Environmental Protection Agency, Berkeley, California, USA; <sup>2</sup>Physicians Institute of Postgraduate Training, Kazakhstan Ministry of Health, Almaty, Kazakhstan; <sup>3</sup>Kazakhstan Ministry of Health, Almaty, Kazakhstan; <sup>4</sup>Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, Washington, DC, USA; <sup>5</sup>United Nations High Commissioner for Refugees, Astana, Kazakhstan; <sup>6</sup>National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, USA

High levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; up to 208 pg/g fat) were measured in samples of breast milk collected in 1997 from 64 donors [41 first-time mothers (primiparae)] living on state farms in southern Kazakhstan. TCDD was the major contributor (70%) to the toxic equivalents, matching the congener patterns found in breast milk and serum samples collected in 1994 and 1996 from donors in nearby villages. The highest TCDD levels were found in state farms adjacent to a reservoir (zone A), which receives agricultural runoff from cotton fields. TCDD levels in zone A were significantly higher than levels in a region more distant (zone B; > 10 miles) from the reservoir (zone A: mean 53 pg/g, n = 17; zone B: mean 21 pg/g, n= 24; p = 0.0017). Levels of TCDD in breast milk and animal-derived foodstuffs were 10 times U.S. levels. Body burden and dietary data suggest that exposures to TCDD are chronic, environmental, and long term and may be related to the use of chemicals in cotton agriculture. The data suggest that the most likely source is the use of cotton defoliants contaminated with TCDD, and the most likely pathway for human exposure is via the consumption of contaminated foodstuffs. Key words: breast milk, coplanar PCBs, dioxins, exposure assessment, furans, Kazakhstan, TCDD. Environ Health Perspect 107:447-457 (1999). [Online 27 April 1999] http://ehpnet1.niehs.nih.gov/docs/1999/107p447-457hooper/abstract.html

In 1994, the first comprehensive investigation of persistent organochlorine contaminants in a country of the former Soviet Union was undertaken. Congener-specific concentrations of polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs), as well as 19 organochlorine (OC) pesticides were measured in breast milk samples collected from first-time mothers (primiparae) living in regions of southern Kazakhstan using the World Health Organization (WHO) protocol (1-3). Samples of infant and adult foods were also analyzed. These studies were initiated because little was known about breast milk contamination in Kazakhstan. Additionally, concerns expressed about milk contamination may decrease interest in breast-feeding and lead to increased birth rates and infant mortality.

High concentrations of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) were found in several breast milk samples taken in 1994 from an agricultural region in southern Kazakhstan (1,2). In 1996, PCDDs and PCDFs were measured in samples of breast milk, serum, and food collected from the same region. Breast milk and serum samples shared a distinctive congener pattern: TCDD was present at high concentrations (10–120 ng/g fat) and was the major contributor (70%) to the international toxic equivalents (I-TEQ) for PCDDs/PCDFs (2).

Breast milk offers a convenient and noninvasive means of assessing concentrations of persistent lipophilic xenobiotics in humans. Several public health and environmental benefits result from monitoring breast milk for persistent contaminants. First, such data provide insight into adult body burdens, historical human exposures, and environmental conditions. Second, these measures complement general environmental monitoring and provide a more accurate assessment of human exposures. Finally, perinatal doses to the fetus and the nursing infant can be estimated.

As a consequence, numerous studies in different countries have been undertaken to measure concentrations of chlorinated contaminants in breast milk as a way of estimating body burdens (1,4-7), and to understand the uptake, distribution, and excretion of these compounds in breast milk. Maternal age and parity are risk factors for contaminant concentrations in breast milk. For example, TCDD levels in breast milk increase ( $\approx 0.1-1$  pg/g per year) with increasing age of the mother (8-12) and decrease with the increasing number of breast-fed children (i.e., parity), roughly 25% for each successive child (8,9,13).

The major pathway for exposure of a residential population to PCDD/PCDF

mixtures is normally via the diet, chiefly through the consumption of contaminated fish, poultry, eggs, milk, and animal fat. Mean TCDD and pentachlorodibenzo-p-dioxin (PeCDD) concentrations in fish are about 10-fold higher than concentrations in foods of animal origin (14). Consumption of contaminated fish from the Great Lakes, Hudson River, northern Canada and Alaska, the Baltic Sea, and the Northern Atlantic is suspected to elevate PCB and OC concentrations, and in some cases PCDD/PCDF concentrations, in subsistence fish-eating populations at these locations above those experienced by other local residents (14,15).

Recent studies have implicated many of these polychlorinated chemicals as potential endocrine-disrupting compounds, or "environmental hormones." Several polychlorinated contaminants have estrogenlike activities, whereas the dioxinlike chlorinated compounds have antiestrogenic activities (16-20). Health effects as diverse as shortened duration of lactation in mothers (21) and neurodevelopmental deficits and intellectual impairment in children (22–25) have been attributed to some of these polychlorinated compounds. For PCDDs/PCDFs, contaminant concentrations in breast milk (8,14,26,27) and human health effects (14,28-30) have been recently reviewed and include, for TCDD, cancer (14,31-40) and altered infant sex ratio (41). The possible adverse effects of body burdens of the dioxinlike polychlorinated contaminants and

Address correspondence to K. Hooper, Hazardous Materials Laboratory, California Environmental Protection Agency, 2151 Berkeley Way, Berkeley, CA 94704 USA. Telephone: (510) 540-3499. Fax: (510) 540-2305. E-mail: kim\_hooper@hotmail.com We express our appreciation to the women of Kazakhstan who volunteered for this study, as well as to the health professionals who assisted in recruitment, interviewing, and sample collection, especially A. Mamaikov. We also express our appreciation for the technical assistance received from G. Lucier, D. Patterson, and W. Turner.

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the PCDDs/PCDFs can be expressed in the mother as well as in potential developmental effects in the infants and children, exposed *in utero* and/or via breast-feeding (42).

PCDDs/PCDFs are produced as persistent unwanted by-products from a variety of industrial and natural processes. These include the chemical synthesis of chlorophenoxyacetic acid herbicides, hexachlorophene, chlorophenols, chlorodiphenyl ether herbicides, and hexachlorobenzene; pulp bleaching in paper mills; operation of cement kilns; and the incineration of municipal waste, sewage sludge, hospital waste, and wood (14). Each process produces a distinct and characteristic PCDD/PCDF congener profile. Because these processes occur in most industrialized countries, residents are exposed to the sum of PCDD/PCDF complex mixtures from the individual processes. The resulting body burden is a cumulative congener profile that is both complex and changeable.

Simpler profiles are seen on occasion in unusual circumstances. TCDD was the predominant congener in some batches of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) produced in the United States (43) and USSR (44) in the 1960s and was a significant by-product of industrial explosions of trichlorophenol (TCP) manufacturing facilities in the United States, Germany, and the Netherlands (14,33,36,45,46).

High TCDD concentrations (15-200 pg/g) are currently found in serum samples from workers with past exposures to industrial accidents or herbicide production in the previously described and other occupational settings (14,33-35,39,40,45). These concentrations are considerably lower than the high TCDD concentrations (1,000-2,000 pg/g) estimated by back-calculation to the time of last exposure. High TCDD concentrations have also been found in a resident population after a TCP workplace explosion. In Seveso, Italy, 16 years after an industrial TCP accident, the mean TCDD concentration was 53-61.5 pg/g in serum taken from donors living in the highly contaminated area (14,47,48).

The high TCDD concentrations (≈ 100–200 pg/g fat) found in breast milk samples from Kazakhstan approach the TCDD concentrations found in serum samples from 2,4,5-T/TCP workers or from residents exposed to chemical catastrophes. In the Kazakhstan agricultural region, however, we have no evidence for an industrial point source or accident as the source of the TCDD contamination.

In 1962–1971 in Vietnam, the U.S. Armed Forces program Operation Ranch Hand aerially sprayed 11 million gallons of Agent Orange, a 1:1 mixture of the defoliants 2,4,5-T and 2,4-dichlorophenoxyacetic acid (2,4-D) over large areas to deprive the enemy

combatants of protective forest cover and food. The 2,4,5-T was heavily contaminated with TCDD (2–3 ppm), about 1,000-fold higher than TCDD concentrations found in domestic phenoxy herbicide products (43,49). High (333–1,832 pg/g fat) concentrations of TCDD were found in breast milk samples collected from residents in the spray areas in 1970. Concentrations had dropped to 2–11 pg/g by 1985–1988 (14,49,50).

In Kazakhstan, the TCDD-contaminated area lies within the fourth largest cotton-growing region in the world. Agricultural chemicals were heavily used in the former Soviet Union and were first applied in Kazakhstan to cotton in the early 1950s. Defoliants are used on cotton. A plausible exposure scenario is that TCDD-contaminated agricultural chemicals were used on cotton fields in southern Kazakhstan.

The highest TCDD concentrations in these studies were found in donors who had lived most of their lives on cotton-growing state farms where, as young women, they routinely picked cotton for 1 month each year. The present study sought to further identify sources of TCDD exposure by collecting breast milk and food samples from 64 primiparous and multiparous (multiple birth) donors from the cotton-growing state farms.

### **Materials and Methods**

Study design. Procedures used in the earlier studies (design, exposure assessment questionnaire, informed consent, PCDD/PCDF target analytes, WHO/EURO protocol for breast milk sample collection, and statistical analysis) were followed (1,2). As per the WHO protocol (51), selection criteria for breast milk donors were primiparae with infants 2–8 weeks of age, with mother and infant in good health.

The exposure assessment questionnaire was modified from the standard WHO questionnaire (51). Our questionnaire determined the health status and breastfeeding pattern of the infant; the health status, food frequency pattern, smoking status, medication use, and residential history of the mother; and work history of the mother and father. Kazak female physicians familiar with the local diet administered the food frequency survey. Frequencies were self-reported and subject to recall bias. The dietary survey should be considered approximate, as no attempt was made to precisely measure or record daily food intake.

Breast milk samples (100 mL) were collected in February 1997 from 64 donors that lived on one of six state farms in the cotton-growing region of southern Kazakhstan, near the rural site of sampling in 1994 and 1996 (1,2). All donors had infants 2–8 weeks of

age; most donors were primiparae (41/64), and the others were multiparae.

Recruitment and enrollment of donors. Study participants were recruited from a list prepared by the Maternal and Child Health Clinic (MCHC) of the roughly 500 infants in the region that were 2–8 weeks of age at the date of sampling. Home births and midwifery do not occur, so all 2- to 8-week-old infants born in the region were listed. Each entry consisted of the names of mother and infant, state farm residence, and mother's reproductive history (parity, numbers of miscarriages, malformed babies, or infant deaths).

We selected six state farms for study based on their high birth and high infant mortality rates (birth rates = 30–40/1,000 population; infant mortality rates = 17–36/1,000 live births). Of the approximately 500 mothers in the region with 2- to 8-week-old infants, 202 lived on one of the six state farms. Fifty-nine of these were first-time mothers. All were recruited. Of the 59 primiparae, 15 were not available for the study (3 could not be located, 8 were temporarily staying with relatives outside the region, and 4 were not breast-feeding). Of the 44 available breast-feeding primiparae, 41 (93%) agreed to participate in the study.

Identifying donors with high TCDD concentrations can produce leads on identifying sources of TCDD exposure. Multiparae can serve this purpose, and a group of multiparae (23 of 143) were selected with adverse reproductive health outcomes (miscarriages, birth defects, or infant deaths) which might signal high contaminant concentrations and help identify major risk factors for TCDD exposure. Multiparae also provide an opportunity to measure TCDD concentrations in older women and, thereby, go back in time to see if TCDD concentrations were much higher in the past. Primiparae are not compared to multiparae. Multiparae were used only to identify major risk factors or to assess concentrations in older women.

We selected primiparae to be representative of younger women in the region: recruitment and enrollment were systematic and complete. In contrast, selection of multiparae was not representative, but attempted to identify those at risk for highest exposures using an abnormal health outcome. Data from the exposure assessment questionnaire for these multiparae were used to identify sources of TCDD exposure.

Zones of contamination. To simplify data analysis, we divided state farms into two regions based on their TCDD concentrations. Three contiguous state farms, whose primiparae had high mean concentrations of TCDD in their breast milk, were placed in zone A. Zone A is located within

10 miles of the southern shore of Lake Chardara, a man-made reservoir that receives runoff from the extensive cotton irrigation systems of Uzbekistan and southern Kazakhstan (Figure 1). Other state farms in Kazakhstan and Uzbekistan were located in zone B, farther south (> 10 miles) of the reservoir's southern shore.

Urban sites are the major cities in southern Kazakhstan (200,000-1 million), distant from the zone A/B geographic region. Rural sites are the two major villages (18,000 and 30,000 population) in the cotton-growing region: one is the county seat of zone A, and the other is the county seat of zone B. Donors from the rural sites are from the same geographic region as donors from zone A and zone B, but they are from villages, not state farms. The two donor populations have different food sources and different socioeconomic levels. The populations of zones A and B, from the cottongrowing state farms, have a lower socioeconomic status than villagers from the county seats, and they grow their own food.

Major residence. We defined major residence as that location where donors lived at least 50% of their lives. It was used in all evaluations of demographic and contaminant data. This was necessary because most study participants had lived in their present residence for < 5 years (primiparae: 30/41 = 73%; and multiparae: 15/23 = 65%) because new brides traditionally move to the village of the husband's family. Thus, current residences are frequently not the major residence. All donors reported having a major residence, and many (44/64) had lived most (> 75%) of their lives on one state farm.

Food. We collected samples of lipid-rich foods, where available, from breast milk donors. Each donor contributed at least one of the following: cow or camel's milk, butter, hard white cheese, lamb fat, or cooking oils (cottonseed, sunflower, and bausak).

Analytical methods. Breast milk and food samples were analyzed by laboratories at the Centers for Disease Control and Prevention (CDC) and the U.S. Food and Drug Administration (FDA), respectively. Breast milk samples were analyzed for PCDDs/PCDFs by high-resolution gas chromatography/isotope dilution high-resolution mass spectrometry based on published methods (52,53). Analytes were separated from samples by a solid-phase extraction procedure followed by a multi column automated cleanup procedure. The analytes were separated on a capillary column and quantified using high-resolution (10,000 resolving power) mass spectrometry capable of selected ion monitoring. Individual analyte concentrations were

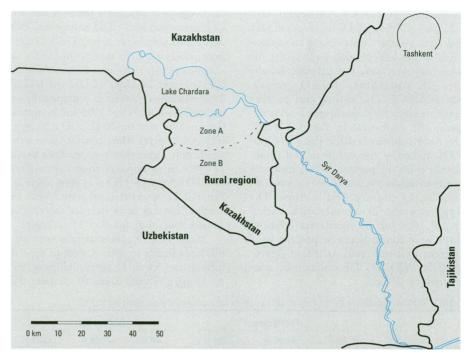


Figure 1. Map of zones A and B in the rural region of southern Kazakhstan.

determined by comparing relative response factors generated using isotopically labeled and known native standard concentrations.

Food samples were spiked with 15  $^{13}C_{12}$ -labeled PCDD/PCDF standards and extracted using a modified Association of Official Analytical Chemists (AOAC) extraction procedure (2:1:1 ethanol:hexane:ethyl ether in the presence of sodium oxalate) (54). Lipids were determined gravimetrically (55); cleanup is described elsewhere (56). Extraction and cleanup of other foods is described elsewhere (57). Extracts were analyzed by quadrupole ion storage MS/MS using multiple reaction monitoring and multiple frequency irradiation (57), modified from the method used by Plomley et al. (58).

Residue concentrations are expressed as picograms per gram milk lipid. For all reported data, I-TEQs for PCDDs/PCDFs and PCBs are based on the WHO-TEQ system (59,60). I-TEQs are calculated from PCDDs and PCDFs.

Statistical analysis. Analytical data were stored in EXCEL 5.0 (Microsoft, Redmond, WA) and ACCESS 97 (Microsoft), and questionnaire data were stored in ACCESS 97. All statistical analyses were conducted in STATA 5.0 (Stata Corp., College Station, TX). Only measurements above the detection level were used in the statistical analyses and are reported in the tables. We examined distributions of each chemical compound for symmetry, multimodality, and normality. Natural log transformation was used for highly skewed distributions. In some cases, log transformation did not achieve normality.

For multiparae, we used a nonparametric (Wilcoxon rank-sum) test to compare congener concentrations. For primiparae, the t-test was used on the nontransformed data because the sample size was large (n = 41).

We examined the correlations between the various compounds by scatter plots and correlation coefficients. After eliminating colinear variables (contaminants), we used multivariate techniques to identify contributors to high exposures (age, diet, etc.). We expected certain exposure factors to emerge as possible determinants of high exposures. We compared the congener patterns in breast milk samples from Kazakhstan with the patterns found in breast milk samples from other countries. Unless otherwise stated, all results reported for women or mothers refer to primiparae.

#### Results

Demographic data. The demographic characteristics of primiparae and infants from zone A were similar to those of infants and donors from zone B and were similar to those of the participants in the 1994 and 1996 studies (Table 1). Most primiparae were Kazak (38/41); 3 were Tajik. There were more male infants than female infants in zone A (12 males, 5 females). Most multiparae (20/23) were Kazak; 2 were Tajiks, and 1 was Russian. Multiparae were significantly older (28 vs. 23 years: p < 0.0001) than primiparae.

Analytical results. The 17 2,3,7,8-substituted PCDDs/PCDFs were measured in breast milk samples from 64 donors. Three

congeners [1,2,3,4,6,7,8-heptaCDF, 1,2,3,4,7,8,9-heptaCDF, and octaCDF] had unacceptable contaminant concentrations in method blanks, and results for these congeners were not reported. Results for 13 congeners, I-TEQ values, pentaCDD/TCDD ratios, and percent lipids are given for milk samples from primiparae and multiparae in Tables 2 and 3. Data are summarized for the 1994 and 1996 studies by region (urban and rural). Results from the present (1997) study are summarized by region in three ways: all samples, by country, and by zone. The lipid content of breast milk varied (1.9-7.1%), but no significant relationships were found between percent lipid content and maternal or infant demographics, PCDD/PCDF contaminant concentrations, or diet.

Geographic differences (zones A and B). Concentrations of TCDD and pentaCDD were significantly higher in breast milk samples from primiparae from state farms (1997) or rural sites (1994, 1996) than from urban areas (1994) (Table 2). Mean TCDD and I-TEQ concentrations measured in primiparae from the rural site in 1994 and 1996 and from state farms in 1997 were similar (TCDD = 35 pg/g; I-TEQ  $\approx$  45 pg/g). These concentrations were significantly higher than concentrations found in 1994 in primiparae from urban sites (TCDD = 5 pg/g; I-TEQ = 11 pg/g; Table 2).PentaCDD concentrations were higher in samples from state farms and rural sites than in samples from urban sites (9 vs. 13 vs. 3 pg/g).

Mean TCDD concentrations were significantly higher in zone A than in zone B (primiparae: 53 vs. 21 pg/g; multiparae: 32 vs. 13 pg/g; Tables 2 and 3), as were the

Table 1. Summary statistics for demographic variables for rural population sampled in 1997.

		Primiparae			Multiparae	
	All	Zone A	Zone B	All	Zone A	Zone B
Ethnicity (n)						
Kazak	38	14	24	20	6	14
Tajik	3	3	0	2	2	0
Russian	0	0	0	1	1	0
Total	41	17	24	23	9	14
Infant's sex (n)						
Male	23	12	11	13	5	8
Female	18	5	13	10	4	6
Total	41	17	24	23	9	14
Maternal age (years)	$23 \pm 5^{a}$	$24 \pm 6$	$23 \pm 4$	$28 \pm 5$	25 ± 2	$30 \pm 6$
n	41	17	24	23	9	14
Mother's BMI <sup>a</sup>	$21.5 \pm 1.8$	$21.0 \pm 2.1$	21.7 ± 1.6	$21.5 \pm 2.2$	$21.3 \pm 1.8$	$21.7 \pm 2.5$
n	39	15	24	22	9	13
Infant's age (days)a	$50 \pm 20$	$49 \pm 19$	$50 \pm 21$	$46 \pm 22$	$52 \pm 23$	$42 \pm 20$
n	41	17	24	23	9	14
Infant's bw (g)a	$3,160 \pm 437$	3,165 ± 447	3,157 ± 439	$3,474 \pm 496$	$3,389 \pm 694$	$3,529 \pm 334$
n	40	17	23	23	9	14

Abbreviations: BMI, body mass index; bw, birth weight.

<sup>a</sup>Mean ± standard deviation.

ranges for primiparae (zone A: 10-208 pg/g; zone B: 4-97 pg/g; Figure 2). TCDD concentrations in primiparae decreased with distance from Lake Chardara: zone A > rural villages > zone B > urban areas (Table 2, Figure 2), which was highly significant in a trend test (p < 0.0001). TCDD concentrations and years of cotton picking are compared for primiparae in zones A and B in Table 4.

The non-TCDD I-TEQ appears to be background because it is relatively constant across the rural/state farm regions in Kazakhstan (Figure 2). Thus, the contribution of TCDDs to the I-TEQ (%TEQ) decreases as one leaves zone A (Table 2; trend test, p < 0.0001). Primiparae whose major residence was in Uzbekistan, a cotton-growing country, and multiparae whose major residence was in Tajikistan, where no cotton is grown, have low TCDD and %TEQ values (Tables 2 and 3).

Concentrations of TCDD, pentaCDD, and hexaCDD congeners were significantly higher in zone A than in zone B. As the congeners become more heavily chlorinated, the difference in concentrations between zones A and B decreases (TCDD > pentaCDD > hexaCDD > heptaCDD > octaCDD; Figure 3). The more highly chlorinated congeners appear to be background contaminants for both zones A and B, whereas TCDD appears to be a major contaminant for zone A (2). As one leaves zone A, therefore, pentaCDD/TCDD ratios increase (zone A, 0.30; zone B, 0.43; rural, 0.52; urban Kazakhstan, 0.51; trend test p < 0.0001; Table 2). These ratios are 10-fold lower than the mean pentaCDD/ TCDD ratio of 2.3 for breast milk samples from other countries (14).

Table 2. Concentrations (pg/g fat) of polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) in breast milk from primiparae.

	By region (1994/1996)				By country (1997) <sup>a</sup>						By zone (1997) <sup>a</sup>				
	Ur	ban (1994)	Rui	al (1994, 1996)	Ali	state farms <sup>a</sup>	K	azakhstan	ι	Jzbekistan		Zone A		Zone B	
Compound	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	п	Mean ± SD	n	Mean ± SD	n	mean ± SD	<i>p</i> -Value <sup>b</sup>
TCDD	32	5.34 ± 3.21	23	34.7 ± 34.9	41	34.5 ± 36.0	32	39.7 ± 38.5	9	16.4 ± 16.2	17	53.4 ± 44.3	24	21.2 ± 21.0	0.0034
PentaCDD	27	$2.50 \pm 1.31$	22	13.1 ± 9.28	38	$9.27 \pm 9.89$	29	10.7 ± 10.9	9	4.72 ± 2.70	15	14.7 ± 13.8	23	$5.74 \pm 3.18$	0.0049
1,2,3,4,7,8-HexaCDD	4	$1.06 \pm 0.51$	22	$3.76 \pm 2.12$	38	$1.55 \pm 2.17$	30	$1.85 \pm 2.34$	8	$0.41 \pm 0.60$	15	2.67 ± 2.97	23	$0.81 \pm 0.94$	0.0078
1,2,3,7,8,9-HexaCDD	8	$0.86 \pm 0.53$	17	$1.78 \pm 1.0$	38	$0.77 \pm 1.15$	29	$0.87 \pm 1.25$	9	$0.42 \pm 0.64$	15	1.29 ± 1.37	23	$0.43 \pm 0.84$	0.0211
1,2,3,4,6,7,8-HeptaCDD	31	$9.95 \pm 5.57$	22	11.4 ± 5.81	38	12.7 ± 36.5	29	14.7 ± 41.7	9	$6.27 \pm 4.89$	15	21.8 ± 57.8	23	$6.73 \pm 5.03$	0.218
OctaCDD	32	102 ± 52.1	22	147 ± 117	40	$29.8 \pm 19.6$	31	$29.2 \pm 16.2$	9	$31.9 \pm 29.6$	17	$95.0 \pm 272$	24	$30.4 \pm 22.0$	0.252
TCDF	19	1.37 ± 1.36	15	$0.58 \pm 0.27$	41	$0.14 \pm 0.41$	32	$0.19 \pm 0.45$	9	$0 \pm 0$	17	$0.25 \pm 0.47$	24	$0.07 \pm 0.35$	0.171
1,2,3,7,8-PentaCDF	- 5	$1.61 \pm 0.83$	16	$0.73 \pm 0.47$	38	$0.09 \pm 0.28$	29	$0.11 \pm 0.32$	9	$0 \pm 0$	15	$0.22 \pm 0.42$	23	$0 \pm 0$	NP
2,3,4,7,8-PentaCDF	31	$5.65 \pm 4.02$	23	$5.24 \pm 2.16$	41	$5.82 \pm 2.26$	32	$5.93 \pm 2.39$	9	$5.43 \pm 1.80$	17	$6.35 \pm 2.70$	24	$5.44 \pm 1.86$	0.205
1,2,3,4,7,8-HexaCDF	31	2.41 ± 1.30	23	$2.43 \pm 0.99$	41	$2.12 \pm 0.99$	32	$2.15 \pm 1.02$	9	$2.02 \pm 0.92$	17	2.44 ± 1.01	24	$1.9 \pm 0.93$	0.0849
1,2,3,6,7,8-HexaCDF	31	$1.90 \pm 1.08$	21	$2.78 \pm 1.41$	38	$2.36 \pm 1.13$	29	2.47 ± 1.18	9	$2.03 \pm 0.96$	15	$2.59 \pm 0.98$	23	$2.22 \pm 1.22$	0.332
1,2,3,7,8,9-HexaCDF	1	0.48	1	0.35	41	$0.37 \pm 1.29$	32	$0.47 \pm 1.45$	9	$0 \pm 0$	17	$0.34 \pm 0.56$	24	$0.38 \pm 1.63$	0.919
2,3,4,6,7,8-HexaCDF	16	$1.07 \pm 0.54$	18	$1.87 \pm 0.90$	35	1.2 ± 1.17	26	1.27 ± 1.31	9	$0.99 \pm 0.67$	12	1.21 ± 0.72	23	1.20 ± 1.37	0.972
I-TEQ <sup>c</sup>	32	$10.8 \pm 5.68$	23	$46.0 \pm 37.0$	40	43.1 ± 40.2	31	48.9 ± 43.2	9	23.0 ± 16.4	16	65.6 ± 50.4	24	28.1 ± 22.2	0.0011
% TEQ <sup>d</sup>	32	$49 \pm 9$	23	$69 \pm 12$	41	72 ± 17	32	$74 \pm 16$	9	63 ± 19	17	77 ± 17	24	68 ± 16	0.0747
PentaCDD/TCDD	27	$0.51 \pm 0.18$	22	$0.52 \pm 0.31$	38	$0.38 \pm 0.22$	29	$0.36 \pm 0.21$	9	$0.44 \pm 0.27$	15	$0.30 \pm 0.19$	23	$0.43 \pm 0.23$	0.0917
% Lipid	32	4.54 ± 1.17	23	5.33 ± 1.77	37	4.41 ± 1.22	28	4.43 ± 1.16	9	4.35 ± 1.56	13	4.03 ± 1.10	24	4.62 ± 1.25	0.163

Abbreviations: I-TEQ, international toxic equivalents for PCDDs/PCDFs; NP, test not performed; SD, standard deviation.

<sup>\*</sup>Major residence in 1997 study. \*Two-sided p-value from t-test indicates if zone A has a significantly different level from zone B. \*Based on PCDDs and PCDFs only. \*100 × TCDD/I-TEQ.

Maternal age. Although no significant correlations were found between I-TEQ or PCDDs/PCDFs and most demographic variables, the concentrations of four penta- and hexaCDD/CDF congeners were significantly correlated with mother's age. PentaCDD and 1,2,3,4,7,8-hexaCDD concentrations were positively correlated with mother's age (r =0.40 and 0.42; p = 0.014 and 0.008, respectively), as were concentrations of 2,3,4,7,8pentaCDF and 1,2,3,4,7,8-hexaCDF (r = 0.66 and 0.39; p < 0.0001 and = 0.012, respectively). 1,2,3,4,7,8-HexaCDD and 2,3,4,7,8pentaCDF were also found in the 1996 study to be positively correlated with mother's age (2). Mean TCDD concentrations and mean years of cotton picking are given for primiparae sorted by age (< 26 years, 26-40 years) and major residence (zones A and B) in Table 4.

Diet. TCDD concentrations in food samples collected from breast milk donors in 1994, 1996, and 1997 are summarized in Table 5. TCDD was found in some vegetable oils (one sample each of cottonseed oil and bausak oil: quantitation limit = < 0.1 pg/g; Table 5). TCDD concentrations for animal-derived food samples, ranked by residence, were in the order state farms > rural villages > urban, the same rank order as breast milk

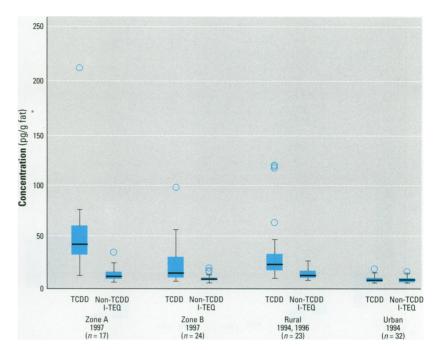


Figure 2. TCDD and non-TCDD international equivalent (I-TEQ) concentrations (pg/g fat) in breast milk samples from primiparae living in zones A and B and in rural and urban areas. Fifty percent of the samples have levels within the box; median, 25th, and 75th percentiles, are shown. Circles indicate observations more than 1.5 box length from 75th percentile.

Table 3. Concentrations (pg/g fat) of polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) in breast milk from multiparae by major residence (1997).

				By country					By zone						
	Α	ll state farms	ŀ	(azakhstan		Tajikistan	ι	Izbekistan		Zone A	Z	Zone B			
Compound	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	<i>p</i> -Value <sup>a</sup>		
TCDD	23	20.4 ± 18.6	17	24.8 ± 19.8	2	3.9 ± 0.14	4	9.5 ± 1.77	9	31.5 ± 22.5	14	13.2 ± 11.6	0.0198		
PentaCDD	21	$7.05 \pm 8.61$	17	$8.06 \pm 9.32$	1	1.4	3	$3.17 \pm 0.46$	9	10.5 ± 12.2	12	$4.48 \pm 3.14$	0.165		
1,2,3,4,7,8-HexaCDD	20	$0.87 \pm 1.0$	16	$1.02 \pm 1.05$	1	0	3	$0.33 \pm 0.58$	8	$0.91 \pm 1.08$	12	$0.83 \pm 0.99$	0.869		
1,2,3,7,8,9-HexaCDD	23	$0.61 \pm 0.96$	17	$0.76 \pm 1.05$	2	$0.55 \pm 0.78$	4	$0 \pm 0$	9	$0.66 \pm 0.81$	14	$0.59 \pm 1.07$	0.578		
1,2,3,4,6,7,8-HeptaCDD	23	8.84 ± 15.3	17	10.4 ± 17.6	2	4.95 ± 2.62	4	$4.0 \pm 2.32$	9	4.56 ± 1.51	14	11.6 ± 19.3	0.508		
OctaCDD	23	$35.2 \pm 47.8$	17	$39.2 \pm 54.8$	2	$0 \pm 0$	4	$0 \pm 0$	9	$19.2 \pm 6.39$	14	$45.6 \pm 59.6$	0.101		
TCDF	23	$0.30 \pm 0.65$	17	$0.35 \pm 0.72$	2	16.6 ± 8.20	4	$27.8 \pm 18.5$	9	$0.20 \pm 0.32$	14	$0.37 \pm 0.80$	0.807		
1,2,3,7,8-PentaCDF	23	$0.18 \pm 0.52$	17	$0.24 \pm 0.59$	2	$0 \pm 0$	4	$0 \pm 0$	9	$0.21 \pm 0.42$	14	$0.16 \pm 0.59$	0.361		
2,3,4,7,8-PentaCDF	23	4.51 ± 1.74	17	4.71 ± 1.83	2	4.4 ± 2.12	4	$3.7 \pm 1.30$	9	$4.26 \pm 1.69$	14	4.67 ± 1.81	0.682		
1,2,3,4,7,8-HexaCDF	23	2.11 ± 1.40	17	$2.24 \pm 1.24$	2	$2.65 \pm 3.75$	4	$1.33 \pm 0.67$	9	$1.82 \pm 0.69$	14	$2.3 \pm 1.72$	0.777		
1,2,3,6,7,8-HexaCDF	23	$2.87 \pm 4.06$	17	$3.22 \pm 4.66$	2	$2.55 \pm 2.19$	4	$4.5 \pm 0.61$	9	$1.93 \pm 0.60$	14	$3.46 \pm 5.17$	0.729		
1,2,3,7,8,9-HexaCDF	23	$0.14 \pm 0.48$	17	$0.19 \pm 0.55$	2	$0 \pm 0$	4	$0 \pm 0$	9	$0.37 \pm 0.73$	14	$0 \pm 0$	NP		
2,3,4,6,7,8-HexaCDF	20	1.96 ± 4.24	15	$2.29 \pm 4.80$	1	4	4	$0.20 \pm 0.40$	7	$0.76 \pm 0.55$	13	$2.61 \pm 5.20$	0.472		
I-TEQ <sup>b</sup>	23	$26.8 \pm 22.7$	17	32.4 ± 24.1	2	$7.34 \pm 1.77$	4	$12.9 \pm 2.74$	9	$39.6 \pm 28.4$	14	18.6 ± 13.8	0.0167		
% TEQ <sup>c</sup>	23	72 ± 12	17	74 ± 12	2	54 ± 11	4	74 ±8	9	79 ±8	14	68 ± 12	0.0508		
PentaCDD/TCDD	21	$0.35 \pm 0.18$	17	$0.35 \pm 0.20$	1	0.37	3	$0.33 \pm 0.07$	9	$0.32 \pm 0.21$	12	$0.36 \pm 0.16$	0.670		
% Lipid	21	$4.61 \pm 1.64$	15	$4.61 \pm 1.69$	2	$5.95 \pm 0.72$	4	$3.96 \pm 1.67$	7	$5.05 \pm 1.57$	14	$4.39 \pm 1.69$	0.456		

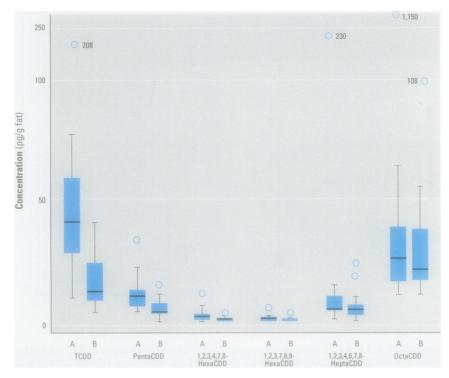
Abbreviations: I-TEQ, international toxic equivalents for PCDDs/PCDFs; NP, test not performed; SD, standard deviation.

Table 4. Years of picking cotton and TCDD concentrations (pg/g fat) in primiparae (mean ± standard deviation).

		All			Zone A	1		Zone B			
Age	n	TCDD	Years picking cotton	n	TCDD	Years picking cotton	п	TCDD	Years picking cotton		
All	41	34.5 ± 36.0	4.8 ± 3.6	17	53.4 ± 44.3	6.6 ± 4.4	24	21.2 ± 21.0	3.5 ± 2.3		
< 26	30	29.5 ± 23.5	$4.4 \pm 3.2$	13	$41.8 \pm 20.0$	$5.3 \pm 3.7$	17	$20.2 \pm 22.2$	$3.7 \pm 2.6$		
≥ 26	11	$48.2 \pm 57.5$	5.9 ± 4.7	4	91.3 ± 79.9	$10.8 \pm 4.4$	7	23.5 ± 19.4	3.1 ± 1.7		
≥ 26 <sup>a</sup>	10	32.2 ± 23.5	$5.8 \pm 4.9$	3	$52.4 \pm 22.1$	12.0 ± 4.4					

Excluding the outlier (208 pg/g fat).

<sup>&</sup>quot;Two-sided p-value from Wilcoxon rank-sum test, indicates whether zone A has a significantly different levels from zone B. Based on PCDDs and PCDFs only. Base100 × TCDD/I-TEQ.



**Figure 3.** Polychlorinated dibenzo-*p*-dioxins (PCDD) congener concentrations (pg/g fat) in breast milk samples from primiparae in zones A and B. Fifty percent of the samples have levels within the box; median, 25th, and 75th percentiles are shown. Circles indicate observations more than 1.5 box length from 75th percentile.

**Table 5.** TCDD concentrations (pg/g fat) in selected foods from state farms, rural villages, and urban areas in southern Kazakhstan.

Region	Food	п	No. of TCDD positives	Percent positive	Mean ± SD	Maximum
State farms	Cow's milk	18	16	95	1.1 ± 1.1	4.9
(1997)	Camel's milk	1	1	100	0.89	0.89
	Butter	11	10	91	$1.6 \pm 2.3$	7.6
	Lamb fat	10	8	80	$0.78 \pm 0.71$	2.1
	Beef fat	1	1	100	0.7	0.7
	Cottonseed oil	2	1	50	0.08	0.08
	Bausak	2	1	50	0.14	0.14
	Total	45	38	84		
Rural villages	Cow's milk	1	1	100	1.8	1.8
(1994/1996)	Butter	6	2	33	0.33	0.52
	Lamb fat	2	2	100	0.52	0.56
	Cottonseed oil	3	0	0	< 0.13	NA
	Bausak	1	0	0	< 0.1	NA
	Total	13	5	58		
Urban	Camel's milk	1	0	0	< 0.1	NA
(1994)	Butter	3	0	0	< 0.1	NA
, ,	Lamb fat	2	1	50	0.12	0.12
	Beef fat	1	0	0	< 0.1	NA
	Total	7	1	14		

SD, standard deviation.

samples. In most (6/7) food samples from urban donors, TCDD was not measurable at detection limits of 0.07–0.2 pg/g fat, whereas measurable TCDD concentrations were found in most (36/45) samples from state farms

Questionnaire data indicated alcohol consumption and cigarette smoking were rare among study participants. Food intake is compared to TCDD concentrations in

breast milk in Table 6. Mean TCDD concentrations were higher in primiparae who had ever eaten (vs. never eaten) fish, lamb fat, kefir, hard white cheese, or beef, although the differences were not significant. TCDD concentrations were significantly higher for primiparae and multiparae who ate fish or chicken from home rather than from a market.

#### **Discussion**

Unusual congener concentrations. TCDD concentrations in zones A and B were unusually high, 10-fold higher than background concentrations elsewhere (Kazakhstan state farms = 35 pg/g; mean of 33 countries = 3.4pg/g) (14). As was true for breast milk samples collected from primiparae in 1994 and 1996 (rural) (2), TCDD was the major (70%) contributor to the I-TEQ in samples collected from state farms in 1997 (Table 2). This is in contrast to the congener patterns found in breast milk samples from 33 other countries, where TCDD contributes 17% of the I-TEO (14). In zone A, the median TCDD concentration exceeds the median concentration for octaCDD (Figure 3). This is very different from values from 33 countries, where the median octaCDD concentration is 50-fold higher than the median TCDD concentration (14).

PentaCDD concentrations in the contamination zone were not unusual compared to concentrations found in other countries. Maximum pentaCDD concentrations were similar to maximum values reported for 33 countries (57 vs. 55 pg/g), as were mean pentaCDD values (zone A = 14.7, state farms = 9.3, rural = 13.1, urban = 2.5 pg/g; mean of 33 countries = 7 pg/g; Table 2). PentaCDD did not constitute an unusually large part of the I-TEQ (Kazakhstan = 4.5/43 = 10%; 33 countries = 3.5/20 =18%) (14). PentaCDD does appear, however, to be part of the distinct congener profile found in the contaminated region because the pentaCDD concentrations in primiparae were higher in zone A, state farms, and rural areas than in the urban areas. Only the TCDD concentrations in samples from the contamination region are unusually high when compared to samples from the rest of the world.

Selection bias. In contrast to the 1994 and 1996 studies, in which participants were volunteers from villages or cities, the 1997 study recruited and accounted for all primiparae with infants 2–8 weeks of age from six state farms. In spite of different recruitment protocols, high TCDD concentrations and high %TEQ values were found in samples from all three studies. There did not appear to be significant bias in the 1997 study for socioeconomic status or infant sex.

Economic status. Some primiparae (12%) had left the region temporarily because of the severe cold and lack of heating. MCHC staff reported that those residents remaining on the state farms were neither richer nor poorer than those leaving.

Infant sex. All (500) infants 2–8 weeks of age that were born in the region were listed by the MCHC. Half of them were male. Thus, the infant sex ratio in the

region appears to be normal. However, the local culture favors male infants over female infants, and a higher proportion of mothers with male infants might be expected to volunteer for the study. If this occurred, more primiparae with male infants would participate in the study than primiparae with female infants. This was not found. The proportion of male infants in the earlier studies (1994 and 1996), where participants were random volunteers, was similar to the proportion in the 1997 study, where nearly every primiparae that satisfied the selection criteria was enrolled (proportion of male infants: 16/27 = 59% in 1994/1996 vs. 23/41 = 56% in 1997). Thus, given the limited sample sizes, there was no evidence for a selection bias for male infants in the 1997 study.

In our earlier study, five of the six primiparae with the highest TCDD concentrations had male infants (2). In the 1997 study, the region of high TCDD concentrations (zone A) also had more male than female infants (12 males, 5 females). However, no significant association was seen between infant sex and TCDD concentrations. Primiparae with high TCDD concentrations (> 30 pg/g; n = 22; 12 males) were no more likely to have male infants or to have female infants than primiparae with low TCDD concentrations (< 30 pg/g; n = 19; 11 males).

Risk factors for TCDD exposure. A multivariate analysis that related TCDD concentrations with relevant risk factors (maternal age, parity, major residence, years of cotton picking, and consumption of selected foodstuffs) was performed. Only major residence (zone A vs. zone B) was a significant risk factor (p = 0.01).

Maternal age. In situations with relatively homogeneous background dietary exposures, TCDD and I-TEQ concentrations clearly increase with age. In the three Kazakhstan studies, concentrations of some penta- and hexaCDD/CDF congeners, but not TCDD, significantly correlated with age of primiparae (2). This suggests that the penta- and hexa- congeners, but not TCDD, are part of a relatively constant background exposure. When primiparae were stratified by major residence (zones A and B) and age ( $< 26, \ge 26$ ), no significant correlations were found between TCDD concentrations and maternal age except for younger (< 26 years) primiparae (r = 0.37; p = 0.04) and younger primiparae in zone B (r = 0.46; p = 0.06). This suggests that TCDD exposures in zone A are less homogeneous than exposures in zone B.

The increase in TCDD concentrations with age in primiparae < 26 years in zone A (4.2 pg/g fat/year) was severalfold higher than rates reported elsewhere. In a study of

Table 6. TCDD concentrations (pg/g fat) in breast milk by food intake and food source.

Food	Intake <sup>a</sup>	n	Mean ± SD	Median	<i>p</i> -Value
Primiparae ( $n = 41$ )					
Fish	Υ	11	$44.3 \pm 59.9$	18	$0.30^{b}$
	N	30	$31.0 \pm 22.4$	26.2	
Lamb fat	Υ	22	42.1 ± 45.1	28.2	0.15 <sup>b</sup>
	N	19	25.8 ± 19.1	25	
Kefir	Y	26	36.1 ± 42.2	23.7	0.72 <sup>b</sup>
	N	15	$31.9 \pm 22.7$	26.3	
Hard white cheese	Y	24	40.1 ± 43.8	31.1	$0.24^{b}$
	N	17	26.6 ± 19.3	25	
Beef	Y	32	$37.9 \pm 39.1$	28.2	0.27 <sup>b</sup>
	N	9	22.6 ± 19.3	18	
All donors $(n = 64)^c$		_			
Fish	Home	13	41.8 ± 55.5	17.7	$0.06^{d}$
	Market	7	$12.5 \pm 9.40$	8.5	
Eggs	Home	30	$32.3 \pm 41.5$	17.6	$0.90^{d}$
-33-	Market	4	24.6 ± 19.2	24.1	
Chicken	Home	21	27.6 ± 44.3	13.6	$0.04^{d}$
	Market	3	$6.6 \pm 2.48$	7.5	2.0.

SD, standard deviation.

112 breast milk donors in Germany, TCDD concentrations increased with age at 0.12 pg/g fat/year (8,9). The increase of I-TEQ with maternal age was also measured in primiparae in Germany using serum samples (180 donors: 0.3 pg/g/year) (10) or using breast milk samples in the Netherlands (41 donors: 1.11 pg/g/year) (11) and Germany (600 donors: 0.63 pg/g/year) (12).

The lack of correlation in zone A between maternal age and TCDD concentrations may have several causes. First, TCDD exposures in zone A may be less homogeneous (e.g., some primiparae may consume large amounts of TCDD-contaminated fish), which may obscure any relationship between TCDD concentration and age. Second, the study has limited power to detect such a relationship, given the small number (n = 11) and narrow agerange of the older ( $\geq$  26 years) primiparae.

Parity and TCDD exit rates. TCDD concentrations were higher in primiparae than in multiparae (mean TCDD = 35 vs. 20 pg/g, p =0.042; Tables 2 and 3). Among older women (≥ 26 years), mean TCDD concentrations decreased as parity increased. Primiparae (n =11) had a mean TCDD concentration of 48 pg/g fat, women with two children (n = 6) had a concentration of 33 pg/g, and women with three or more children (n = 7) had 15 pg/g. These are similar to the decreases with increasing parity found in breast milk studies in Germany (8,9). In these studies, TCDD concentrations in breast-feeding mothers (n = 112) decreased 27% and 47% with second and third children, respectively (8,9). Mean PCDDs/PCDFs concentrations in women nursing their second child (n = 74) were 20-30% lower than concentrations in primiparae (n = 79) (13).

Three primiparae donated breast milk samples in December 1996 and in February 1997. TCDD concentrations in samples from two of the women decreased 33% (from 21 to 14 pg/g fat) over the 3-month interval, in agreement with other studies that show major decreases in TCDD concentrations early in lactation (6 weeks, 15%; 12 weeks, 25%) (8,9). The rate of elimination for the two women is 2.3 pg/g fat per month of breast-feeding. For the third woman, the TCDD concentration decreased 90% over the 3-month period (from 103 to 11 pg/g fat), corresponding to an elimination rate of 30 pg/g fat per month of lactation. We do not have an explanation for this apparent high rate of elimination. Clearance rates of TCDD and PCBs increase with decreasing body mass index (61) and increasing body burdens (62), respectively, and either of these may contribute to the different exit rates observed. Breast milk samples from the three women had similar lipid content (2%). In our earlier studies, two women donated breast milk and serum samples 2 years apart. Their TCDD off-loading rates (long-term lactation) are estimated at 4-5 pg/g fat per month of breast-feeding (2).

Geographic location. Location (major residence) was a major risk factor for PCDD/PCDF contamination, with higher contamination in zone A. Primiparae in zone A were 10 times more likely to have high TCDD concentrations (> 30 pg/g) than primiparae in zone B (p = 0.0016). A primiparous donor from zone A had the highest TCDD concentration (208 pg/g).

The populations from zones A and B, both cotton-growing regions, appear to have been exposed to the same PCDD/PCDF

<sup>&</sup>lt;sup>a</sup>Y, ever eaten (at least once a month); N, never eaten. <sup>b</sup>p-Value from t-test for mean difference between "ever eaten" and "never eaten." <sup>c</sup>Primiparae and multiparae (not all donors indicated food source). <sup>d</sup>p-Value from Wilcoxon rank-sum test for median difference between "home" and "market."

congener mixture, but at different concentrations. The PCDD congener profiles of samples from zones A and B are similar, but the concentrations are 3-fold higher in zone A than in zone B (Table 2).

The populations in the urban areas appear to have been exposed to a different PCDD/PCDF congener mixture. The congener profile found in zones A and B is different from the PCDD congener profiles found in samples from the urban regions.

TCDD concentrations in samples from the urban areas are similar to the mean in 33 other countries (5 vs. 3.4 pg/g), although TCDD contributes more to the I-TEQ (50% vs. 17%) (14). Concentrations of dioxinlike non-TCDD PCDDs/PCDFs and coplanar PCBs are lower in urban Kazakhstan than in western industrialized countries

Cotton agriculture. Cotton was first grown in the region in the early 1950s, and heavy use of agricultural chemicals soon followed. Aerial application of pesticides and defoliants occurred during the period 1965–1985. Defoliants are applied to cotton in late August, just before harvest. Temperatures reach 40°C in summer, and surface volatilization of applied organic chemicals is likely to be substantial.

The PCDD/PCDF congener patterns seen in breast milk samples collected in 1994, 1996, and 1997 resemble those found in the TCDD-contaminated herbicide and defoliant 2,4,5-T, and in Agent Orange (2,4,5-T and 2,4-D). Although TCDD-contaminated stocks of 2,4,5-T and 2,4,5-trichlorophenol were produced in Russia in the 1960s (44), there are no records of their use on cotton in this region. The only evidence that these agents were used is the distinctive congener profile of PCDD/PCDF contaminants in breast milk samples, which is similar to the signature pattern of PCDDs/PCDFs (TCDD is the predominant congener) characteristic of contaminated 2,4,5-T.

For the past 40 years, the region's cotton crop has been harvested by local teenagers, 14–18 years of age. Women reported being sprayed while picking cotton or while at home. They reported overspray incidents involving entire state farm communities, which were followed by outbreaks of headaches and nausea.

If TCDD-contaminated chemicals were used in cotton agriculture, residents of state farms would be chronically exposed to TCDD over a number of years. Exposures would be via multiple pathways (e.g., inhalation of contaminated dust and volatiles, skin contact with leaves and soil, and ingestion of contaminated food). Once the state farm region is contaminated with TCDD, the residents would likely

be continually contaminated because all their food, including vegetables, milk, eggs, and meat, is grown on the state farm.

Primiparae in zone A have twice the number of years of cotton picking as same-age primiparae in zone B (Table 4), suggesting that cotton agriculture was more intense in zone A than in zone B. TCDD concentrations are higher in breast milk samples from zone A than samples from zone B. The different body burdens may be due to heavier use of agricultural chemicals in zone A.

Cotton picking. If TCDD were a contaminant in a cotton pesticide or defoliant, the number of years of cotton picking would be a risk factor for exposure. Supporting this, mean years of cotton picking were higher in zone A than in zone B (6.6 vs. 3.5 years, p = 0.003), as were the mean TCDD concentrations (Table 4).

Whether women picked cotton during the period of aerial spraying (1965-1985) could also be a risk factor. Older women, who are now 26-40 years old and were teenagers before 1985, would have picked cotton during this period. If aerial spraying were a significant source of exposure, older women should have higher TCDD concentrations than younger women, although the 10-year difference in mean ages of the younger and older groups (22 vs. 32 years), about 1.5 TCDD half-lives, would work against this. Older primiparae (age 26-40 years), in fact, do have higher TCDD concentrations than younger primiparae (< 26 years; 48 vs. 30 pg/g, p = 0.07), especially in zone A (91 vs. 42 pg/g, p = 0.02; Table 4).

However, the higher TCDD concentrations in these older women may have resulted from picking cotton for a longer time, not from picking cotton during 1965–1985. Older primiparae in zone A, in fact, picked cotton twice as long as younger primiparae (11 vs. 5 years; Table 4), and years of cotton picking positively correlated with age (r = 0.44, p = 0.0046). In zone B, where both age groups picked cotton for the same number of years, mean TCDD concentrations were the same (24 vs. 20 pg/g).

On closer examination, neither the duration of cotton picking nor the period during which cotton was picked seem to be risk factors. In zone A, although the mean TCDD concentration for younger women is lower than that of older women (42 vs. 91 pg/g; Table 3), this difference disappears if the outlier (208 pg/g) is removed from older primiparae (42 vs. 52 pg/g). The difference also disappears if TCDD values are ageadjusted to 26 years, using TCDD changing 4.2 pg/g fat/year of age (59 vs. 66 pg/g).

Combining donors from zones A and B, no correlations were seen between TCDD concentrations and years of cotton

picking either in primiparae (r = 0.27, p = 0.09) or in multiparae (r = 0.09, p = 0.67) or between TCDD concentrations and years of cotton picking during 1965–1985 (primiparae only).

Finally, six donors who had never picked cotton had elevated TCDD concentrations. These included primiparae (zone A = 58 and 42 pg/g; zone B = 26 and 9 pg/g) and multiparae (zone A = 32 pg/g; zone B = 14 pg/g). It is clear that mothers can have exposures to TCDD without picking cotton.

Use of agricultural chemicals. Suggestive evidence that agricultural chemicals are a source of TCDD exposure comes from a tractor driver/sprayer of pesticides/herbicides on cotton fields who had high TCDD concentrations. Comparing TCDD concentrations in multiparae 36 years and older, a 40-year-old mother of six children had a TCDD concentration of 50 pg/g, quite high for her age and parity (Table 7). Of all study participants, she was the only one who reported working with farm chemicals.

Correlations that would further support the link between TCDD concentrations and exposures to agricultural chemicals were not found. Although primiparae in zone A had more years of cotton picking and higher TCDD concentrations than primiparae in zone B, no correlations were seen between TCDD concentrations and number of years of picking cotton (stratified by zones A and B), or the number of years picking during the aerial spray period. The small size of the population limited the power of the study to detect such correlations.

Cotton agriculture in Kazakhstan. Outside of zone A and the Kazakhstan cotton-growing region, TCDD concentrations in breast milk samples were lower. At urban sites distant from zone A, TCDD concentrations in samples from primiparae averaged 5 pg/g (Table 2) (2). Concentrations were also low (< 10 pg/g, n = 10) in non–cotton-growing areas near zone A, including seven nearby non–cotton-growing state farms, as well as Chardara, a city located 20 miles away in a non–cotton-growing region. They were lower still in samples from two multiparae

**Table 7.** TCDD concentrations in women 36–40 years of age.

		No. y	ears	No. years picking cotton in zone		1
Age	Parity	Α	В	Α	В	TCDD (pg/g)
36	5	15	21	22	0	17
37	5	0	37	0	0	14
37	6	0	37	0	26	9
40a	6	0	40	0	6	50
36	1	26	10	15	2	75
40	1	31	9	0	9	31

<sup>&</sup>lt;sup>a</sup>Tractor driver.

whose major residence was a non-cotton-growing country (Tajikistan: 4 pg/g; Table 3), about 100 miles away.

TCDD concentrations in samples from nine primiparae and four multiparae whose major residence was in the cotton-growing regions of Uzbekistan 20 miles away were also low (16 and 10 pg/g, respectively). We do not have an explanation for this. Despite our inquiries, we obtained little information about comparative cottongrowing practices in Kazakhstan and Uzbekistan. The differences in TCDD concentrations observed in samples from cotton-growing regions of Kazakhstan and Uzbekistan could arise from the differences in agricultural practices or the use of different agricultural chemicals. Alternatively, the TCDD contamination in Kazakhstan could arise from nonagricultural sources.

*Diet.* State farm residents in zones A and B grow most of their own food. In contrast, the residents of the rural villages in zones A and B buy most of their food from sources outside the cotton-growing region.

The dietary staples of cow's milk, lamb fat, and butter appear widely contaminated in state farms. In most of the animal-derived food samples from state farms (36/41), TCDD was present at measurable concentrations. These concentrations were roughly 10-fold higher than concentrations measured in two U.S. samples by the same laboratory (butter, lamb fat: 0.06 pg/g).

A few food samples had TCDD concentrations that were 5-fold higher than the mean (e.g., cow's milk, 4.9; lamb fat, 2.1; butter, 4.3 and 7.6 pg/g fat), making the median, rather than the mean, more representative of the central tendency. Median TCDD concentrations were 1.0, 0.33, and 0.6 for cow's milk, lamb fat, and butter, respectively. TCDD concentrations were not significantly different in food samples from zone A and zone B.

TCDD concentrations in food samples followed the same order as breast milk samples: state farms > rural > urban. The food samples with high TCDD concentrations did not come from donors with high TCDD concentrations in their breast milk. Given the long half-life of TCDD and the recent change in residence for most primiparae, this is understandable. Exposures to TCDD may have occurred years ago via consumption of food that was more contaminated than similar foods today. Also, local fish may be a major source of exposure, but samples were not available for analysis. Animal fats contributed an estimated 20-40 pg TCDD/day to TCDD exposure, as calculated from questionnaire data on intake frequencies and from lab measurements of TCDD residues in cow's milk, lamb fat, and butter.

TCDD concentrations were significantly higher for primiparae and multiparae who ate fish or chicken from home rather than from a market. Chicken from home is probably more heavily contaminated than market chicken because TCDD concentrations are more likely to be higher in the local backyard soils in the cottongrowing region than in the soils in the chicken-growing state farms that are 40-50 miles away. Similarly, home fish may be more contaminated than market fish. TCDD concentrations may be higher in sediments in the local canals or in the portion of Lake Chardara adjacent to the cotton-growing state farms than in the sediments of the remainder of Lake Chardara or downstream regions of the Syr Darya River, 60–100 miles away.

Donors who consume home fish or chicken may have lower socioeconomic status and a more contaminated environment or workplace. There is no evidence to date that diet is the source of the higher TCDD concentrations found in breast milk samples from zone A because zones A and B had similar patterns of food consumption (residue levels and intake frequencies). However, zone A residents may consume more fish from Lake Chardara, the catchment basin for agricultural runoff adjacent to zone A.

Most women reported eating cottonseed oil every day. A low TCDD level has been found in cottonseed oil (57) but not in surface soil samples collected from cotton storage bins in 1994 (1).

Dose to infants and young adults. Using standard exposure models, the TCDD concentration in a 2-year-old breast-fed infant is calculated to be 7-fold higher than the concentration in her mother. Thus, a 2year-old infant girl (11 kg) who is breast-fed for 2 years by a zone A primiparous woman (mean TCDD concentration = 53 pg/g fat) is estimated to have a TCDD concentration of 340 pg/g fat. This estimate assumes that TCDD concentrations in infants at birth are 25% the maternal concentrations (63), that the ingestion rate of a nursing infant is 0.7 L/day (51), that infants absorb 90% of the ingested TCDD (64), and that the halflife of TCDD is 4 years in infants 0–2 years

The daily intake of TCDD for a typical woman in zone A was estimated using a model that assumed a chronic, long-term environmental exposure at a constant level. In this model, an infant grows up to have the same body burden as her 18-year-old mother. A TCDD input of 175 pg/day is required to transform the 2-year-old daughter (11 kg; 340 pg TCDD/g fat) into an 18-year-old primiparous woman in zone A (55 kg; 52 pg

TCDD/g fat), assuming a half-life of 8 years. This is roughly 10-fold higher than TCDD intake concentrations in the United States and severalfold higher than the intake (20-40 pg/day) estimated from consumption of animal fats in zone A. Using TCDD concentrations measured in food, consumption of 100-200 g animal fat/day (nonfish) is estimated to provide an intake of 175 pg TCDD/day. This seems unlikely, given the mean serving sizes and intake frequencies of these foods in the region. These animal fats (excluding fish) may not be the major source of TCDD exposure. Alternatively, TCDD concentrations are declining, and the daughter's concentration in the future will be significantly lower than that of her mother.

TCDD concentrations in primiparae in zone A increase 4.2 pg/g fat/year between the ages of 18 and 26 years. Assuming 55 kg body weight and 25% body fat, an intake of 20–40 pg TCDD/day would give an increase of 0.5–1 pg/g fat/year. This suggests that there are other major TCDD exposure pathways or that the estimated increase of TCDD per year is inaccurate because of small sample size (n = 17).

An important source of TCDD intake may come from eating TCDD-contaminated fish. Donors from zone A report that they eat fish from Lake Chardara during the summer. If fish had TCDD contaminant concentrations of 70 pg/g fat (10 pg/g wet weight), a reasonable dietary consumption of fish of 100 g (1/4 lb) per week would provide an intake of 164 pg TCDD/day, about 95% of the target intake concentration. Fish from Chardara were not available for sampling during the winter collection trips.

Breast-feeding continues to be beneficial, given the nutritional advantages and the protection against infectious diseases it provides to the breast-fed infant. The I-TEQ of the PCDD/PCDF contaminant mixture in Kazakhstan breast milk is twice that of breast milk in the United States. This is because non-TCDD PCDD/PCDF and coplanar PCB congeners make significant contributions to the TEQ in U.S. samples, whereas these are minimally present in zone A samples. It is not clear what, if any, adverse effects these TEQ concentrations will have on the infants and children in the region.

The highest priority is to identify significant exposure pathways so that future exposures can be prevented. As consumption of fish can be a major exposure pathway, fish from Lake Chardara need to be examined.

## **Conclusions**

In southern Kazakhstan, large numbers of people have high body burdens of TCDD. A large rural population has mean TCDD

concentrations similar to those now found in residents of Seveso's highest zone of contamination and in chemical workers in 2,4,5-T or TCP production facilities who were exposed in the past via TCDD-contaminated feed-stocks or industrial accidents.

A cotton-growing region of southern Kazakhstan is significantly contaminated with TCDD. Women from the region of high contamination (zone A) have greater mean number of years of cotton picking than do women from the region of lesser contamination (zone B). Zone A is adjacent to a large catchment basin for agricultural runoff.

Primiparae from cotton-growing state farms have high TCDD concentrations (35 pg/g fat; range 6–208 pg/g; n=41) compared to primiparae from distant urban areas (urban site: 5 pg/g fat; range 1–16 pg/g fat; n=32). Primiparae from zone A have twice the TCDD concentrations of primiparae from zone B (53 pg/g, n=17 vs. 21 pg/g, n=24; p=0.0017), were 10 times more likely to have high TCDD concentrations (> 30 pg/g) than primiparae in zone B (p=0.0016), and picked more cotton.

TCDD body burdens are low outside the Kazakhstan cotton-growing region: in nearby (< 30 miles) non-cotton-growing state farms in Kazakhstan (mean = 10 pg/g, n = 14), in state farms in Uzbekistan, a major cotton-growing country (mean = 16 pg/g, n = 9), or in Tajikistan, where no cotton is grown (mean = 4 pg/g, n = 2). The TCDD congener pattern is similar to TCDD-contaminated herbicide. TCDD is the major contributor (70%) to the I-TEQ in breast milk samples collected from the contaminated region. TCDD is the predominant congener in TCDD-contaminated batches of the defoliants 2,4,5-T, or Agent Orange.

Cotton defoliants may be the source of TCDD exposure. Defoliants have been applied to cotton fields in Kazakhstan since the early 1950s, with aerial application during 1965–1985. TCDD-contaminated 2,4,5-T stocks were produced in the USSR in the 1960s, and may have been used in Kazakhstan.

The TCDD levels in breast milk in zone A are 10-fold higher than U.S. levels. The I-TEQ is twice that of U.S. concentrations. Dioxinlike non-TCDD PCDD/PCDF and coplanar PCB congeners that make significant contributions to the total TEQ in western industrialized countries are less significant in Kazakhstan.

The food chain is widely contaminated. TCDD was detected in most samples of cow's milk (n = 16), lamb fat (n = 8), and butter (n = 10), but less in vegetable oils used in cooking (cottonseed and bausak oils; n = 2). As with breast milk samples, TCDD

concentrations in animal-derived foodstuffs are 10-fold higher than concentrations measured in U.S. samples. Present dietary intake data, which do not include local fish consumption, do not account for the contamination concentrations that are measured.

TCDD exposure appears to be chronic, environmental, and long term. Data on historical use of agricultural chemicals, and on TCDD concentrations in older women and in food, suggest that this region has a chronic, long-term, environmental exposure to high concentrations of TCDD, the first documented exposure of this kind. We continue to evaluate the sources of the TCDD exposures and to assess the effects of these exposures on the health of women, infants, and children.

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